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Studies of trace elements in biological systems by energy dispersive X-ray fluorescence (EDXRF) and proton induced X-ray emission (PIXE) methods

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Abstract: Applicability of EDXRF and PIXE techniques for trace elemental analysis in biology and medicine is demonstrated. Due to increasing importance of the need to determine the role of essential and toxic trace elements in human health and disease, the method of PIXE analysis has assumed great importance in recent years. This method has been found to be particularly useful for biological samples. EDXRF also offers a complimentary method particularly in the range of elements of Z - 45 to 60 where the sensitivity of PIXE analysis is not quite adequate. EDXRF can also be usefully employed for other elements of the periodic chart with relatively lesser sensitivity. The EDXRF and PIXE facilities set up in our laboratory have been employed for carrying out trace element studies of a variety of biological and medical samples. The work being presented here include trace element analysis of normal and cancer bearing tissues of Swiss mice, trace element profiles in cancerous human oesophageal tissues, investigations on the effect of toxic metals such as Hg from Ayurvedic drugs on Wister rats, and investigations of blood lead levels of children admitted to Sion Hospital from Dharavi slums of Bombay. The results of these investigations are presented and discussed.

Keywords: Trace element analysis, biological applications, EDXRF and PIXE methods.

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1. Introduction

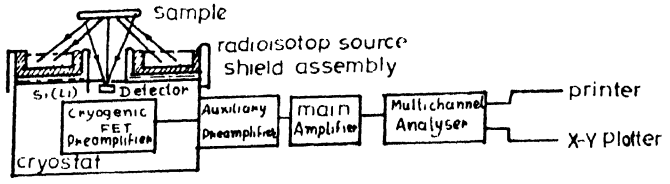
EDXRF and PIXE methods are finding increasing use in biological and medical sciences. The main application is the determination of major, minor and trace elements present in various types of samples, such as tissues, blood and the medical formulations. Trace elements play important role in the cell activity, the formation of skin, muscle, bone and blood. Through food and medicine, they cause chemical reactions that lead to proper digestion, the absorption of nutrients from food and production of hormones. Their deficiency in food is known to cause a variety of diseases. Some of these trace elements known to be essential are Cr, As, Co, Cu, F, I, Fe, Mn, Mo, Ni, Se, Si, Sn, V and Zn and the other essential

major elements are C, H, O, N, S, Ca, P, K, Na, Cl and Mg totalling to twenty six essential elements. There are other elements which are known to be present in animal body and are not essential. These are acquired in the body as environmental contaminants. Amongst these only Hg, Pb and Cd are known to be toxic even at relatively low concentrations. The role of essential or toxic elements is greatly influenced by the chemical environment provided by the other co-existing elements. Therefore methodologies based on multielement analysis such as PIXE and EDXRF can provide extensive information to determine the correlations of various elements present in the biological system. A large number of applications (Andres *et al* 1987, Badica *et al* 1984, Beguin *et al* 1987, Duflov *et al* 1987, Galuszka *et al* 1984, Hall *et al* 1984, Lapatto 1984, Tanaka *et al* 1987, Tapper *et al* 1987, Uda *et al* 1987, Van Rinsvelt *et al* 1986, Wei *et al* 1987, Williams 1987) of PIXE and EDXRF methods in biological and medical sciences have been carried out in recent years. The present work includes studies in the role of trace elements in cancer, toxic elements from environmental pollution and toxicity of Ayurvedic drugs.

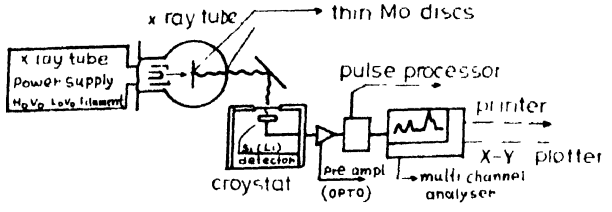
2. Experimental set-up

Schematic diagrams of the experimental set-up (Lal *et al* 1981, Lal *et al* 1985) for the excitation of samples by both photons or charged particles are shown in Figure 1 (a, b, c). The common part of each set up is a high resolution Si(Li) X-ray detector to detect the X-rays of sample materials excited by either photons or charged particles. Photons are obtained from either radioisotope sources (Figure 1(a)) or from a low power X-ray tube (Figure 1(b)). The detector is shielded from direct source radiations and the geometry shown in the figure is optimised to obtain minimum scattered background. Common radioisotopes used are Fe^{55}, Cd^{109} and Am^{241} and for X-ray tubes, a transmission type of air cooled X-ray tube of maximum anode current of 1.0 ma at 50 kV with Mo or Rh targets is used. Figure 1(c) shows a schematic arrangement of the PIXE facility set-up at Trombay. The PIXE chamber is coupled to the beam from 5.5 MeV Van de Graaff accelerator. A set of 12 samples can be mounted inside the evacuated PIXE chamber at a time and analysed using the proton beam of 2.0 to 4.0 MeV energy. The beam from the accelerator is diffused by passing through thin Ni foil located $\sim$ 0.5 m from the sample target. The diffused beam passes through a set of collimators before striking the sample over a well-defined area. The beam current on the target is kept at a level of a few tens of nanoamperes to avoid overheating of the samples and also to limit the count rate in the detector to reasonable values. The X-rays from the sample are detected at 90° with respect to the beam by Si(Li) detector. The absorbers in front of the detector Be window serve the purpose of reducing the low-energy bremsstrahlung background count rate in the detector. The Si(Li) detector is shielded against the proton-induced γ-ray background from all sides, except for where the window interfaces the

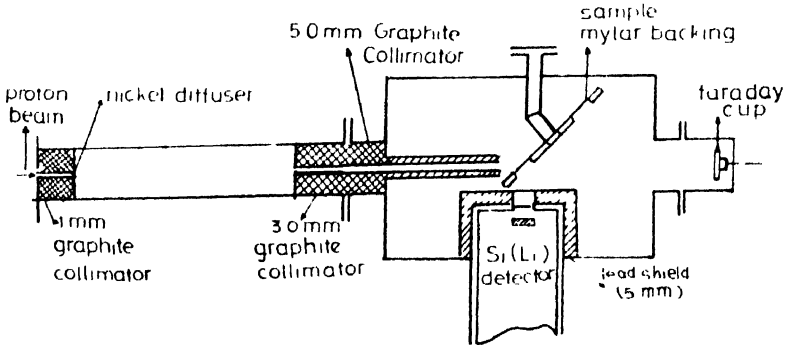
sample and the detector. A Faraday cup is provided at the end to monitor the beam current.



a) Schematic diagram of X-Ray spectrometer and experimental arrangement with radioisotop source excitation



b) Experimental setup with low power X ray tube excitation



c) Exprimental set up of proton beam excitation

Figure 1. Schematic arrangements of EDXRF and PIXE experimental set-up.

3. Sample preparations

The methods of sample preparation employed for EDXRF and PIXE are quite simple. In EDXRF, the samples ground to fine particle size are mixed and homogenised with a binder material (cellulose) and pelleted. Thin sample pellets are employed to reduce the matrix effects and to minimise scattered background from the sample. In PIXE method the sample is acid digested and the solution is micropipetted on a thin (3.6 μm) mylar foil and dried under infra-red lamp. For quantitative calibration a known quantity of a noninterfering element such as Yttrium is added to the sample solution to serve as an internal standard.

4. Minimum detection limits

In EDXRF method minimum detection limits depend largely on the scattered background from the sample. Optimisation of geometry (Lal *et al* 1987) and use of thin samples are the important factors to obtain high sensitivity of detection. We have shown that in this way in the region of $45 \leqslant Z \leqslant 69$ it is possible to attain detection limits in the range of hundred nanograms or less by using the side source geometry of disc source of Am^{241}. In the PIXE method, detection sensitivity ot a few ngs can be attained for $Z < 45$ and for $Z > 70$ using K X-rays and L X-rays respectively and by employing thin samples on very thin backing. In spite of high bremstrahlung background, PIXE method provides better detection limits for low atomic number elements ranging from $Z = 11$ to $Z = 30$ due to high excitation cross sections. In this way EDXRF method in combination with PIXE can give good detection sensitivity for all elements of $Z \geqslant 11$. For still lower atomic number elements, detection is possible only with a window-less detector by mounting both the sample and source inside the evacuated cryostat.

5. Method of quantitative analysis

In case of analysis of thin samples by EDXRF method, the concentration of an element m_j (gm/cm^2) of the element j is related with the X-ray line intensity I_j by a simple expression

$$I_j = I_0\, G.K_j.m_j.C_j$$

where I_0 is the intensity of exciting source and G is the geometrical factor between source, sample and detector. K_j is the relative excitation-cum-detection factor given by

$$K_j = \tau(1 - 1/J_{KL}).\omega_{K,L}.f.T.E \qquad (1)$$

where

τ = total photoelectric cross section (cm^2/gm),

$J_{K,L}$ = Jump ratio for K or L absorption edge,

$\omega_{K,L}$ = fluorescence yield for vacancies giving rise to X-rays from K or L levels,

f = ratio of the intensity of a particular line to be measured with respect to the total intensity of that level,

T = fractlon of X-rays transmitted by the media between sample and the detector,

ε = detector efficiency of the X-ray line to be measured and

$$C_j = \frac{1 - \exp\left(-(\mu_1 \operatorname{cosec} \theta_1 + \mu_2 \operatorname{cosec} \theta_2).m\right)}{(\mu_1 \operatorname{cosec} \theta_1 + \mu_2 \operatorname{cosec} \theta_2).m} \qquad (2)$$

where μ_1 and μ_2 are total mass absorption coefficient (cm^2/gm) of the sample for the exciting and characteristic X-rays respectively. θ_1 and θ_2 are the angles formed

by the exciting and characteristic radiations with the specimen surface, m is the mass of the sample (gms/cm^2), μ_1 and μ_2 for a thin sample can be determined experimentally by transmission method. The factors of K_j can be computed using the values of various constants from literature and by determining the detector efficiency experimentally. Using a sample of known value of m_j, the calibration factor I_0G can be determined for the unknown elements of any sample.

In the case of excitation by proton beam in PIXE method, in a similar manner, the X-ray line intensity $I_x(K_\alpha)$ of an element is related to the mass thickness (t) of the element in μg/cm^2 as

$$I_x(K_\alpha) = 6.24 \times 10^6 \frac{qtN}{M \cos\theta} \cdot \sigma(K_\alpha). \frac{\Omega}{4\pi}.\varepsilon.T \qquad (3)$$

where

q — the cumulative charge in microcoulombs,

θ — angle between target normal and beam direction,

N Avogadro number,

M — atomic wt. of the element,

σ K_α X-ray production cross section (cm^2),

Ω solid angle,

ε = detector efficiency and

T = fraction of X-rays transmitted through sample mounting chamber window, detector window and absorber if used between sample and detector.

For quantitative analysis, one can either calculate calibration constants, or one can use a multi-element standard for internal calibration. For calibration of the geometrical factor, one may employ either an internal standard or an external standard. In the case of external standard the beam intensity is to be determined by Faraday Cup.

6. Data analysis

The X-ray spectrum in both methods of excitation consist of K and L X-ray lines of different elements riding on a continuum background. The background in the case of EDXRF can be represented by a second order polynomial while the background in the case of PIXE in lower energy region is quite complex and the spectrum can be split into parts to represent the background by polynomial functions. The X-ray peak shapes deviate from the gaussian towards the lower energy side. In relative measurements, this error can be neglected. The least square analysis code (XANAL) is employed to obtain the intensities of X-ray lines where overlapping takes place due to limited resolution of the detector. The other factors employed in the equations for quantitative analysis are stored in the code to evaluate the concentrations of elements present in the sample. For evaluation of the geometrical

factors and source dependent constants, it is necessary to employ either external or internal standards in both these methods.

7. Biological/medical applications

7.1. *Trace-element studies of tissues of cancer bearing animals :*

In order to study the role of trace element in the growth of cancer, analysis of trace elements was carried out (Lal *et al* 1986 a) in spleen, liver, kidney, heart and

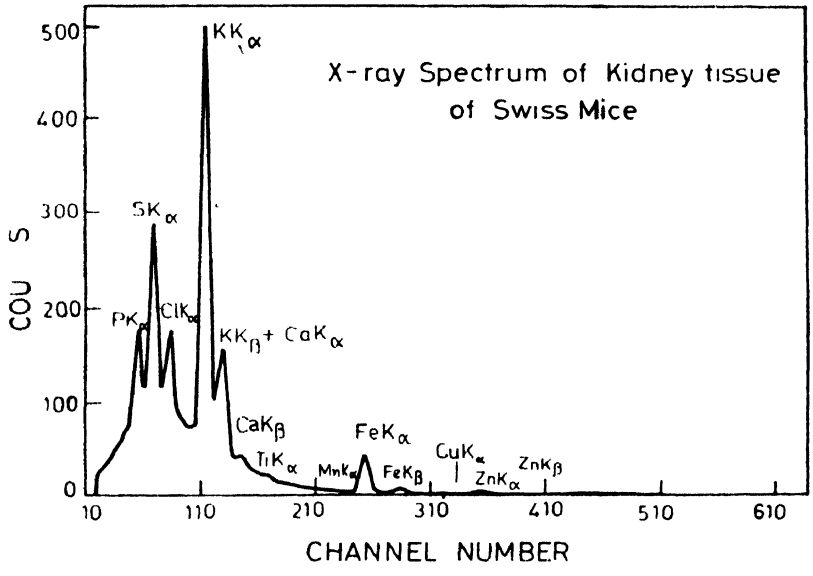


Figure 2. X-ray spectrum of kidney tissue of cancer bearing Swiss mice.

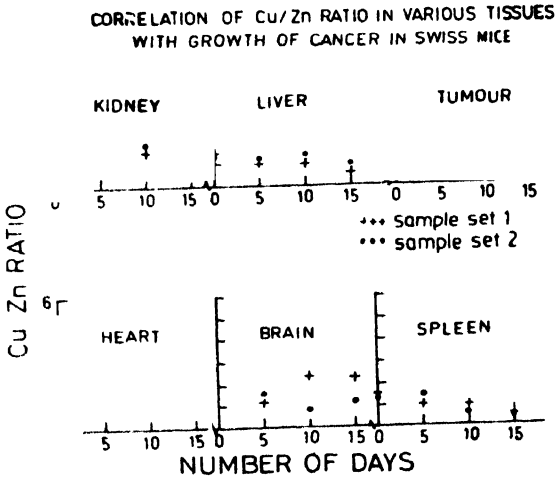


Figure 3. Ratio of Cu and Zn present in tissues of different organs of Swiss mice.

brain of the healthy and cancer bearing swiss mice. The tumour fibrosarcome was transplanted on the chest wall of swiss mice and samples of the tissues of the various organs were collected after every five days. A total of 48 samples were studied to observe the variation of Cu Zn ratio in these tissues with the growth of cancer. Figure 2 shows a typical spectrum of kidney tissue indicating the various elements present. The correlation of Cu Zn ratio in the tissues from normal to progressive growth of cancer in swiss mice over a period of 15 days was studied for two sets of samples. Figure 3 shows the variation pattern of Cu/Zn ratio for the six organs. As seen Cu Zn ratio decreases in the tissues of heart, spleen and liver while this ratio remains constant in kidney and brain.

7.2. *Trace-element profile in cancerous human oesophageal tissues :*

Trace element concentrations were determined (Lal *et al* 1989) in surgically resected, cancerous human oesophageal tissues. Each sample was divided into six zones, E_2 and E_3 formed the central tumour region, E_1 and E_4 were proximal to the oesophagus and E_5 and E_6 distal to the oesophagus. Maligancy was confirmed histopathologically in all cases. Figure 4 shows a typical X-ray spectrum of

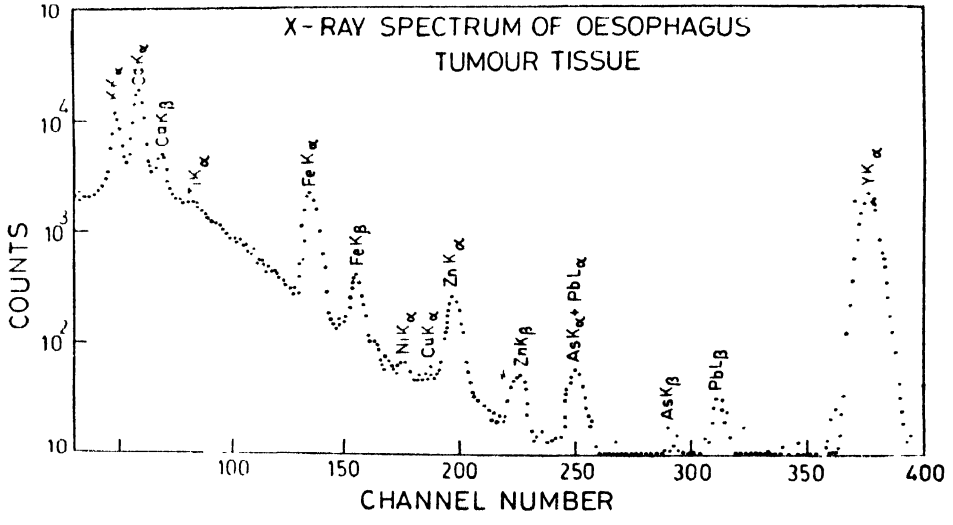


Figure 4. X-ray spectrum of human oesophageal tumorous tissue.

oesophageal tumourous tissue indicating various elements present by the PIXE method. Table 1 shows the quantitative analysis results indicating the mean values and range of different zones (E_1 to E_6) of Ti, Fe, Cu, Zn and As. The trace element profile of these elements from E_1 to E_6 of the above elements are shown in Figure 5. The changes in the concentrations of these elements could be considered to reflect the corresponding physiological status of the transition from normal to intermediate (malignarey associated changes) to neoplastic cells.

7.3. *Investigations of toxic metal (Hg) in Ayurvedic drugs 'Arogyavardhini'* :

Many therapeutic drugs in the Ayurvedic system of medicine (Govt. of India, 1978) are administered, containing large number of elements known to be toxic,

Table I. Mean values and range (in brackets) of concentrations of trace elements in the involved and uninvolved zones of oesophagus.

Elements	Concentrations (μg/gm) of wet weight					
	E_1	E_2	E_3	E_4	E_5	E_6
Titanium	1.0 (0.3 - 2.0)	1.28 (0.4 - 2.6)	0.79 (0.3 1.4)	0.64 (0.2 - 1.0)	0.67 (0.3 - 1.2)	0.94 (0.4 - 1.8)
Iron	12.3 (4.9 - 15.0)	10.9 (6.0 - 10.0)	7.86 (2.6 - 19.2)	7.3 (2.2 - 13.4)	6.9 (2.7 - 13.2)	12.4 (2.3 - 22.0)
Copper	0.215 (.03 - .23)	0.42 (.07 - .43)	0.19 (.04 - .34)	0.32 (.05 - .8)	0.9 (.12 - 1.06)	0.95 (.02 - 4.6)
Zinc	5.5 (3.0 7.83)	5.88 (2.3 - 8.7)	4.6 (1.7 - 8.3)	4.1 (1.6 - 7.1)	6.3 (2.8 - 8.2)	5.6 (1.6 - 5.0)
Arsenic	1.2 (0 18 3.9)	1.47 (0.54 - 1.98)	0.93 (.24 - 2.4)	0.5 (0.28 - 0.84)	0.88 (0.6 - 1.22)	1.92 (0.3 - 3.2)

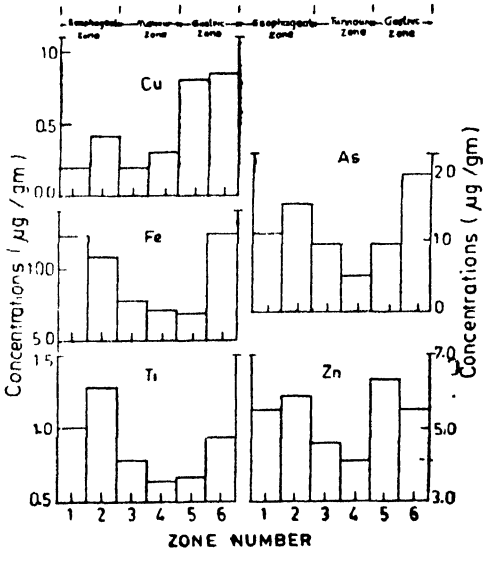


Figure 5. Trace-element profile of human oesophageal tumour tissue in different zones (E_1 to E_6).

as food supplements to treat various ailments. It is essential to study the toxicity of these drugs by measuring the accumulation of the toxic elements in various organs of the biological system treated by these drugs. The wister rats

were chosen to be treated by this drug. This drug obtained from a few manufacturers was analysed by EDXRF method. Figure 6 shows a typical X-ray spectrum

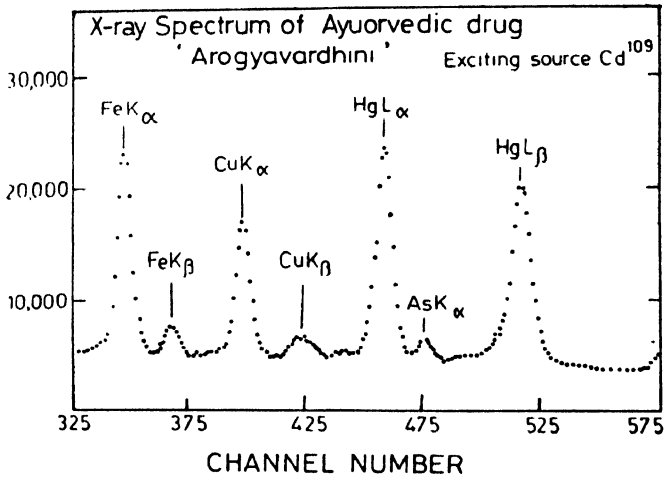


Figure 6. X-ray spectrum of Ayurvedic drug Arogyavardhni.

of this drug indicating the presence of Fe, Cu and Hg. Table 2 shows the concentrations of these elements present in the samples of this drug obtained from different manufacturer. As seen in the table the proper standardisation of these drugs is not ensured by all the manufacturers. The drug administered was

Table 2. Element concentrations (%) of Arogyavardhni drug.

Manufacturer \ Sample No.	Fe				Cu				Hg			
	1	2	3	Av:	1	2	3	Av:	1	2	3	Av:
B	1.8	2.1	1.4	1.77 ±0.3	0.7	0.8	0.7	0.73 ±.05	1.6	1.8	1.4	1.6 ±.16
D	0.9	0.8	1.1	0.93 ±.12	0 2	0.2	0.2	0.2 ±0.0	1.5	1.4	1.6	1.5 ±.08
M	1.8	4.3	1.6	2.57 ±1.23	1.0	2.3	0.9	1.4 ±.64	1.3	3.3	1.2	1.93 ±.97
S	1.8	2.5	2.2	2.17 ±.29	0.4	1.2	0.5	0.7 ±0.36	1.4	1.2	1.5	1.37 ±.12
Z	1.1	1.1	1.0	1.07 ±.05	0.4	0.4	0.4	0.4 ±00	0.8	0.8	0.7	0.77 ±.05
Govt. approved				2.26 (Loh bhasam)				2.26 (Tamrabhasm)				2.26 (Parad)

chosen taking into consideration the minimum deviation of concentrations from sample to sample. The wister rats were fed with this drug of about 40 mg per day

(about 1 mg of Hg per day) for 5 days in a week for 4 months as per the following schedule.

(1) Continuously treated for four months

(2) Treated for 3 months and discontinued

(3) Treated for the fourth month only

(4) Control

Tissues of the animals studied after the above period of administration of drug were kidney, liver, stomach, intestine, heart and Adrenal glands. Figure 7 shows typical X-ray spectrum of one of these tissues of heart sample treated for

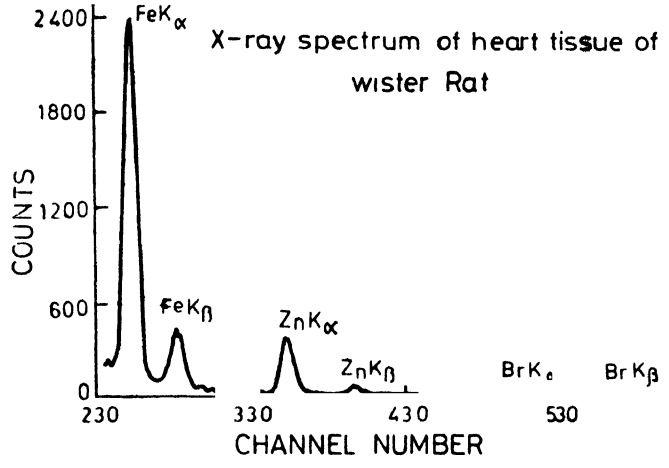


Figure 7. X-ray spectrum of heart tissue of Wister rat fed on Arogyavardhni drug.

one month. Hg was not found to be present in any of the PIXE spectrum of the tissues analysed (Lal *et al* 1986b). These negative results were confirmed by another method of analysis-atomic absorption spectroscopy.

7.4. Investigations of blood lead levels of children :

Studies of blood lead levels of children admitted to Sion Hospital, Bombay from the adjoining slum areas of Dharavi, have been carried out by the Proton-induced X-ray Emission Spectroscopy. Children are known to be particularly sensitive to lead toxicity due to increased absorption and retention of lead. The children of Dharavi slum belong to lower socio-economic class and are also continuously exposed to air pollution due to heavy vehicular traffic and industries in this area. The group in the present study consisted of forty three (43) patients and controls between the age group of 1-12 years and of both sexes. As per clinical findings, the patients were divided into five high rish groups viz. (1) Hypochromic microcyptic anemia group, (2) Gastro-intestinal group, (3) Encephalopathy of unknown aetiolgy, (4) Mental retardation and (5) Pica. Figure 8 shows a typical

X-ray spectrum of the blood sample of one of these patients. The spectrum shows the Pb *L* X-rays in addition to other elements normally present in a blood sample. Quantitative analysis of Pb concentrations (0.2 μg/ml to 6.0 μg/ml) in all the five

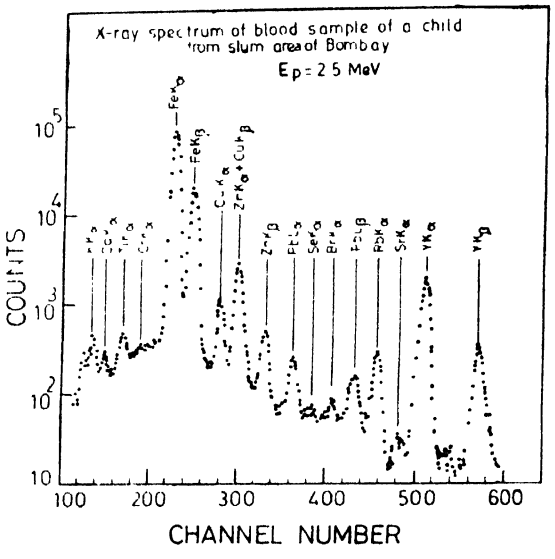


Figure 8. X-ray spectrum of blood sample of a child patient from Dharavi slum.

groups with symptoms of lead poisioning showed high incidence of blood lead levels. This study has shown that pica is one of the most likely source of Pb due to the contamination of soil and dust with Pb particles from automobile exhaust. Some of the controls also showed high lead levels with no symptoms of Pb poisoning. Although these controls were from apparently healthy children from the same area, the air pollution could be the main cause of some of these control samples showing high blood lead levels. This study reveals that high level of the Pb poisoning results from the automobile traffic in urban areas. Table 3 shows the results of analysis of other elements normally present in the blood from forty three samples of both patient and control groups. It is seen that the average values of concentrations of trace elements of children with no lead poisoning (Pb level $< 0.3\mu$ g/ml) compares well with the normal values compiled by G. V. Iyenger (Iyenger *et al* 1978). But trace element concentrations of patients affected by Pb poisoning are in general lower than those of healthy children.

8. Conclusions

In the preceding discussions, it has been clearly demonstrated that PIXE and EDXRF techniques have important role to play in relevant research problems of trace element studies in biological/medical sciences. It is important to emphasise here the role of inter disciplinary research teams consisting of physicist, biologist and medical specialists for carrying our such studies. In order to arrive at firm

Table 3. Concentrations of trace-elements of whole blood of children from slum area of Bombay.

Sample No	Concentrations (μg/ml)						
	Fe	Cu	Zn	Se	Br	Rb	Pb
1	189	1.1	4.7	0.04	0.23	- -	0.97
2	376	1.0	5.1	0.10	0.03	0.16	0.82
3	297	1.1	16.9	0.09	0.44	0.12	0.93
4	170	1.3	2.9	0.11	0.44	0.19	0.12
5	442	0.6	7.9	0.08	0.13	1.96	0.29
6	217	0.7	3.6	0.7	0.24	1.38	0.36
7	286	1.4	3.6	—	1.42	2.77	0.26
8	539	0.6	4.8	0.10	0.12	2.00	—
9	256	2.3	2.9	0.07	3.4	2.18	0.29
10	322	1.9	4.8	0.091	0.39	1.45	0.97
11	568	1.5	4.8	—	0.26	2.11	0.6
12	225	1.6	5.7	—	1.25	1.9	0.2
13	378	0.9	5.8	0.19	-	1.42	4.35
14	429	0.5	7.2	0.19	0.12	1.94	5.0
15	584	0.9	5.9	0.11	0.18	2.46	6.0
16	279	1.3	7.2	0.11	0.76	2.24	3.1
17	326	1.7	5.0	0.13	0.15	1.68	1.5
18	505	1.1	7.5	0.03	0.12	2.92	2.6
19	284	0.9	34	0.05	0.13	2.96	0.7
20	190	0.9	4.1	0.03	0.10	1.78	2.2
21	622	0.8	4.1	—	0.11	2.34	2.32
22	382	1.31	4.1	—	0.17	2.0	2.34
23	241	0.50	2.74	—	—	1.7	1.4
24	225	0.70	4.8	—	0.24	1.1	2.4
25	193	0.70	4.3	0.03	0.22	1.3	2.2
26	329	0.80	6.8	0.02	0.10	1.9	0.7
27	483	1.24	4.2	0.12	0.24	6.2	2.0
28	187	0.40	2.2	—	0.13	0.8	0.5
29	163	0.15	4.4	0.09	0.41	2.0	0.5
30	91	1.77	1.4	—	0.18	0.8	0.3
31	378	0.71	5.4	0.08	0.15	2.4	0.8
32	456	0.84	5.8	—	—	2.1	0.3
33	268	1.01	5.2	0.1	—	1.6	0.8
34	280	2.34	2.9	0.11	—	0.9	5.6
35	760	1.35	6.1	—	—	3.6	0.6
36	344	3.16	7.73	0.18	0.27	2.0	1.4
37	454	2.72	4.4	0.14	0.43	2.84	0.7
38	465	1.08	3.3	0.13	—	1.52	—
39	527	1.30	5.5	—	0.34	2.7	0.23
40	725	1.35	6.0	0.28	0.17	3.4	0.2
41	454	0.71	5.0	0.03	—	1.8	5.5

Table 3. (Contd.)

Sample No.	Concentrations (μg/ml)						
	Fe	Cu	Zn	Se	Br	Rb	Pb
42	629	1.31	8.0	—	0.28	3.3	—
43	478	0.74	5.0	—	0.57	3.0	—
Average	372	1.19	5.2	0.1	0.39	1.97	0.80
Average of healthy	533	1.32	6.3	0.14	0.35	2.57	<.3
International average	447	1.01	7.0	0.171	4.7	2.49	0.21

conclusions regarding the role of trace element levels in various types of studies, one needs biological insight as well as analytical accuracy. These studies are now being extended to include many other medical problems.

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